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Regulation of Sucrose Synthase Expression in *Chenopodium rubrum:* Characterization of Sugar Induced Expression in Photoautotrophic Suspension Cultures and Sink Tissue Specific Expression in Plants

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Summary

The effect of source/sink modifications on the enzyme activity and on the steady state level of mRNA of sucrose synthase has been analyzed in *Chenopodium rubrum.* A sucrose synthase cDNA with high homology to sucrose synthases from both monocotyledonous and dicotyledonous plants has been cloned and used as a homologous probe for Northern blot analyses. Photoautotrophic suspension culture cells, which may be shifted to mixotrophic growth by adding sugars, have been used as a model system to investigate the transition between autotrophic and heterotrophic growth. The higher activity of sucrose synthase after preincubation in the presence of D-glucose, D-fructose or sucrose correlates with an elevated level of mRNA. The sucrose synthase gene was fully induced above a concentration of 20 mM glucose and the steady state level of mRNA was already elevated 1 h after the addition of glucose. Induction of sucrose synthase by 6-desoxyglucose suggests that the non-phosphorylated glucose is the signal for the sugar induced gene expression. Both the enzyme activity and the mRNA level showed a sink tissue specific distribution in plants. The data suggest that sucrose synthase is important both for sink metabolism and the source sink transition.

Key words: Chenopodium rubrum L., sink metabolism, sucrose synthase, sugar induction.

Introduction

During the development of higher plants a purely heterotrophic embryo differentiates into a mosaic of assimilate exporting (source) and assimilate importing (sink) tissues. The relative sink strength of a specific plant organ is not strictly determined and may change during organ and plant development. During the vegetative growth period roots and young leaves are strong sinks whereas with the begin of flower development most of the assimilates are directed into the developing seeds, roots and tubers. The transition of a specific plant organ between carbon heterotrophy and carbon autotrophy is best understood with leaves (Turgeon, 1989).

Although the aim of classical plant breeding is to increase sink strength of harvestable sinks like fruits and roots and thus improve yield, the regulation of source/sink transition on the molecular basis is not yet understood. It is known that several photosynthetic genes are light regulated (Benfey and Chua, 1989), but it remains to be determined which are the molecular signals that induce and sustain heterotrophic metabolism even in the presence of light. There are several indications that hormones are involved in regulating enzymes involved in heterotrophic metabolism (Kuiper, 1993). The role of sugars in gene regulation is well established in both pro- and eucaryotic microbial systems (e.g. regulation of the *lac* operon of *E. coli* and carbon catabolite repression in yeast) and in animal cells (Lin and Lee, 1984). There is growing evidence that also in lower and higher plants sugars do not only serve as carbon source for heterotrophic growth but also function as molecular signals for gene regulation

(Williams et al., 1992; Farrar, 1991; Hilgarth et al., 1991; Koch et al., 1992).

In most plant species sucrose is the main transport sugar and sucrose metabolizing enzymes expressed in sink tissues are likely to be important for sink function. There are two sucrose cleaving enzymes in plants, invertase and sucrose synthase, and especially sucrose synthase has been considered to be the key enzyme of metabolism in sink organs and sucrose synthase activity has been suggested to be used as biochemical marker for sink strength (Claussen et al., 1985; Sung et al., 1989; Wang et al., 1993). Sucrose synthase (E.C. 2.4.1.13) catalyses the conversion of sucrose to UDP-glucose and D-fructose. Although the reaction is readily reversible, the kinetics of sucrose synthase of different plant species have indicated that its primary metabolic role is the breakdown of sucrose. Sucrose synthase is found in all plant tissues and shows the highest activities in sink organs. This enzyme has been suggested to be involved in such diverse pathways as starch biosynthesis (Chourey and Nelson, 1976), cell wall component synthesis (Maas et al., 1990), phloem transport (Claussen et al., 1985), fruit development (Nolte and Koch, 1993), energy generation (Huber and Akazawa, 1986), phloem function (Geigenberger and Stitt, 1993) and root nodule nutrition (Verma and Delauney, 1988). In addition to the regulation by environmental factors (Salanoubat and Belliard, 1989; Martin et al., 1993) specific sugar responses have been reported (Koch et al., 1992; Martin et al., 1993; Karrer and Rodriguez, 1992; Heim et al., 1993).

In order to understand the function of sucrose synthase in source/sink regulation and in sink metabolism the regulation of this enzyme in response to sugars was analyzed in photoautotrophic suspension culture cells and the tissue specific expression was determined in plants. *Chenopodium rubrum* was chosen since a photoautotrophic, hormone and vitamin independent suspension culture of undifferentiated cells, established by Hüsemann and Barz (1977; Hüsemann, 1981), is available which may be shifted to mixotrophic growth by the addition of sugars. This culture seems especially suited to determine the function of sucrose synthase in sink metabolism, since the analysis is not complicated by the presence of different types of specialized cells, which may express specific sucrose synthase isoenzymes with other cellular functions (see above). In contrast to the incubation of cut plant organs in sugar solutions, the experiments using suspension culture cells are not complicated by wounding and anaerobiosis, which were shown to influence sucrose synthase expression (Salanoubat and Belliard, 1989; Martin et al., 1993). The use of a photoautotrophic suspension culture for the analysis of sugar effects obviates the need for a starvation period prior to the experimen, which excludes effects due to energy deprivation.

The sucrose synthase activity of *C. rubrum* has been determined both from sugar treated suspension culture cells and from source and sink tissues from intact plants. To be able to compare physiological and molecular data on sucrose synthase function, the sucrose synthase cDNA from *C. rubrum* has been cloned and sequenced. The observed increased expression of the gene above a basal level in the presence of sugars in the suspension culture system and the sink tissue specific expression in plants correlate with the corresponding enzyme data. The induction by sugars has been further characterized and, based on the observed induction by a glucose analogue, the possible signal transduction pathway leading to the induction of sink metabolism by sugars is discussed.

Materials and Methods

Plants, cell cultures and bacterial strains

The photoautotrophic, hormone and vitamin independent suspension culture of *Chenopodium rubrum* L. (fat hen or goosefoot; Hüsemann, 1981) is a derivative of the culture established by Hüsemann and Barz (1977) and has been subcultured in our laboratory since 1991. The cells were grown as described previously (Roitsch and Tanner, 1994). Photomixotrophic growth was initiated by adding D-glucose, sucrose or D-fructose to the medium. *Chenopodium* plants were grown in the greenhouse under natural light conditions.

The *E. coli* strain DH5α was used for cloning into plasmid and the *E. coli* strain C600 for work with the lambda phage.

Sucrose synthase activity assays

To assay sucrose synthase activity in plant suspension culture cells were washed twice with MS Medium (Murashige and Skoog, 1962) and resuspended at 50% packed cells in extraction buffer (50 mM Hepes-NaOH, pH 7.5; 1 mM DTT; 20 mM KCl, 10% (v/v) glycerol, 0.1% (w/v) BSA). The cells were disrupted by two passages through a French pressure cell (American Instrument C., Silver Spring, MD, USA) at 97 MPa (14.000 psi). Tissue from *Chenopodium* plants was homogenized in a small volume of extraction buffer using a mortar and pistil in the presence of liquid nitrogen. To remove cell debris the extract was centrifuged at 10,000 × g for 5 min at 4 °C. The resulting supernatant (cytosolic fraction), containing 5 to 10 mg protein × mL^{-1} and referred to as «crude extract», was immediately used for enzyme assays. The enzyme preparation was dialyzed against distilled water for 3.5 h at 4 °C. Sucrose synthase activity was tested in the direction of sucrose synthesis essentially as described by Ou-Lee and Setter (1985). A glucose test kit (Boehringer Mannheim GmbH, Mannheim, Germany) was used according to the manufacturer's instructions to determine the amount of glucose liberated. Assays without D-fructose were used as controls. Extract protein contents were determined according to Bradford (1976) with bovine serum albumin (BSA) as the standard protein. The content of soluble protein per g fresh weight (FW) in the different tissues was determined; the corresponding values are 14 g/kg FW in leaves, 5 g/kg FW in stems, and 3.7 g/kg FW in roots.

Specific activity of sucrose synthase is reported as nmol sucrose synthesized × mg^{-1} protein × h^{-1}. The data shown in the paper are the mean values from at least 5 independent experiments.

Invertase activity assay

The intracellular invertase actvities at pH 4.5 and pH 7.0 were determined according to Roitsch et al. (1995). Specific activity of invertase is reported as nmol sucrose hydrolyzed × mg^{-1} protein × h^{-1}. The data shown in the paper are the mean values from at least 5 independent experiments.

Isolation of RNA

For the isolation of nucleic acids cells were harvested by centrifugation, frozen in liquid nitrogen and ground with a mortar and pes-

tle in the presence of liquid nitrogen. Total nucleic acids were isolated according to Bell et al. (1986) and total RNA was isolated by using Nucleobond AX columns (Macherey-Nagel, Düren, Germany) according to the instructions of the supplier.

Construction of a cDNA library and screening

Polyadenylated RNA (5 µg) isolated from glucose incubated suspension culture cells was used to construct a cDNA library using a cDNA-synthesis-kit (Pharmacia Biotech Europe, Freiburg, Germany) according to the instructions of the supplier, except for the use of a CL-4B sepharose column for the separation of non-linked adapters. The cDNA was cloned into Lamdagt10 using a commercial packaging kit (Promega Corporation, Madison, Wisconsin, USA). The library was screened using standard procedures (Sambrock et al., 1989) with the radiolabled fragment from plasmid pB10a (Salanoubat and Belliard, 1989) coding for a sucrose synthase from potato. Three positive clones were identified that were further purified. The complete cDNA insert of one positive clone was subcloned as 3.0 kb NotI fragment and the coding region was subcloned as a 2.2 kb EcoRI into pUC19 (Yanisch-Perron et al., 1985) resulting in plasmids pSS7 and pSS1, respectively.

Sequencing

Nucleotide sequencing using the dideoxy chain-termination method (Sanger et al., 1977) was performed by using the Sequenase 2.0 kit (United States Biochemical Corporation) with subcloned fragments and custom made oligonucleotide primers for sequencing of internal sequences. Sequence analysis was performed using the sequence analysis software package of the University of Wisconsin genetic computer group (UWGCG; Devereux et al., 1984) on a VAX microcomputer.

Northern blot analysis

Total RNA was separated on a denaturating formaldehyde agarose gel and blotted onto reinforced nitrocellulose (Parablot NCL; Machery-Nagel, Düren, Germany). Hybridisation was carried out in the presence of 50 % formamide, 2× SSC, 0.1 % SDS, 5× Denhard solution, and 0.1 mg × mL^{-1} salmon testis carrier DNA at 42 °C. Filters were washed with increasing stringency, the final wash step was carried out with 0.2× SSC and 0.1 % SDS at 56 °C for 30 min. Isolated DNA fragments used as hybridization probes were labeled with (α-^{32}P-dATP by oligolabeling (Feinberg and Vogelstein, 1983).

Results

Activity of sucrose synthase after addition of sugars to photoautotrophic suspension culture cells

The effect of mixotrophic growth on sucrose synthase activity of *C. rubrum* suspension culture cells was tested. A photoautotrophic culture was split into two parallel cultures in the late logarithmic growth phase; 100 mM D-glucose was added to one of the cultures and incubation was continued for 48 h. The second culture was maintained under photoautotrophic conditions and used as control. The suspension culture cells were homogenized by 2 passages through a French press cell and the sucrose synthase activity was determined in the dialysed cytosolic fraction. Addition of 100 mM D-glucose resulted in a significantly increased sucrose synthase activity; the activity was 310 % higher than in control cells maintained photoautotrophically (Table 1). In further experiment the effect of preincubation with D-fructose and sucrose was tested. The activity was 300 % and 390 % higher than in the control cells. To rule out unspecific induction due to the osmotic effect of the sugars applied, the suspension culture cells were incubated in the presence of mannitol; Table 1 shows that the addition of 100 mM mannitol did not result in a significantly increased sucrose synthase activity. To further demonstrate the specific effect of the sugars applied on sucrose synthase activity, the activity of two other cytosolic enzymes, acidic and neutral invertase, was determined. In contrast to the sucrose synthase activity both soluble invertase activities were not affected by the addition of glucose (Table 1).

Table 1: Activity of sucrose synthase and soluble invertases of *C. rubrum* suspension culture cells and different tissues of *C. rubrum* plants.

Cell type	Specific activity (nmol sucrose × mg^{-1} protein × h^{-1})		
	sucrose synthase	soluble invertases	
		pH 4.5	pH 7.0
Suspension culture cells			
autotrophic	117 (24)	2.0 (0.14)	1.7 (0.10)
Mannitol*	147 (19)	n.d.	n.d.
D-Glucose*	361 (28)	2.2 (0.11)	1.6 (0.09)
D-Fructose*	351 (24)	n.d.	n.d.
Sucrose*	458 (14)	n.d.	n.d.
Plants			
Source leaves	140 (6.4)	0.4 (0.03)	0.3 (0.02)
Stems	585 (48)	0.2 (0.01)	0.3 (0.02)
Roots	446 (43)	0.4 (0.03)	0.5 (0.03)

* The cells were incubated for 48 h with 100 mM of mannitol or the sugars indicated. The results shown are the means of at least 5 independent experiments; standard deviations are given in parenthesis. n.d. = not determined.

Distribution of sucrose synthase activity in different tissues of C. rubrum plants

Since the sucrose synthase activity of photoautotrophic suspension culture cells was affected by the shift to mixotrophic growth, the activity of sucrose synthase was tested in source and sink tissues of *C. rubrum* plants. Fresh tissue was homogenized by grinding with a mortar and pestil, and the sucrose synthase activity was tested in the cytosolic fraction. Table 1 demonstrates that sucrose synthase activity could be detected in all tissues analyzed and that the activity shows a sink tissue specific distribution. Whereas the activity was low in source leaves, 140 nmol sucrose × mg^{-1} protein × h^{-1}, the activity was 320 % higher in roots and 420 % higher in stems. Recovery experiments using mixed extracts ruled out the possibility that the lower activity in leave extracts was due to a specific loss of enzyme activity, for example, because of a higher content of secondary metabolites like phenolics or tanins. The sink tissue specific distribution of sucrose synthase activity is further supported by the de-

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TTCATTTTCTTTCATTCATTCATTTATCTATTTTTTTGTTAAAAAATTGTCTGAAAATTA      60
CCGGAAAAACAAGAAATGGCAGGTCGTTTGACGAGGGTTCCGAGCCTCAAGGAGCGTTTG     120
                M  A  G  R  L  T  R  V  P  S  L  K  E  R  L
GATGAAACTCTCACTGCTCAACGCAATGAAATTCTCTCCTTCTTGTCCAGGATTGTGAGT     180
D  E  T  L  T  A  Q  R  N  E  I  L  S  F  L  S  R  I  V  S
CATGGAAAAGGGATTTTGCAACCCCACGAATTGTTGTCAGAATTTGAAGCTGTTTCTGAC     240
H  G  K  G  I  L  Q  P  H  E  L  L  S  E  F  E  A  V  S  D
AAACACAAACTTGCTGATGGCCCTTTTGGTGAAGTTTTTAGGCACACCCAGGAAGCAATT     300
K  H  K  L  A  D  G  P  F  G  E  V  F  R  H  T  Q  E  A  I
GTGTTGCCTCCATGGATCACCCTTGCTGTTCGTCCGAGGCCTGGTGTATGGGAGTACATT     360
V  L  P  P  W  I  T  L  A  V  R  P  R  P  G  V  W  E  Y  I
CGTGTCAATGTTGATGAACTCGCTGTTGAGGAATTGACCCCTTCCCAGTTCCTCCATGTG     420
R  V  N  V  D  E  L  A  V  E  E  L  T  P  S  Q  F  L  H  V
AAGGAGGAATTAGTTGATGGAAGTGTGAATGGAAACTTTGTGCTCGACGAGCTCGATTTT     480
K  E  E  L  V  D  G  S  V  N  G  N  F  V  L  D  E  L  D  F
GAGCCATTCAATGCTTCATTTCCCCGCCCTACCCTCTCCAAATCAATTGGAAATGGTGTG     540
E  P  F  N  A  S  F  P  R  P  T  L  S  K  S  I  G  N  G  V
GAGTTCCTTAACAGACACCTTTCTGCTAAGATGTTCCATGACAAGGAGAGTATGCGCCCG     600
E  F  L  N  R  H  L  S  A  K  M  F  H  D  K  E  S  M  R  P
CTGCTTGACTTCCTTAGGATGCACCACTACAAGGGCAAGACAATGATGCTGAATGACAGA     660
L  L  D  F  L  R  M  H  H  Y  K  G  K  T  M  M  L  N  D  R
ATCCAAAACCTTGATTCTCTCCAAAGTGTACTAAGAAAAGCAGAGGAGTTCCTGGCTACA     720
I  Q  N  L  D  S  L  Q  S  V  L  R  K  A  E  E  F  L  A  T
CTTCCTGCTGACACACCCTACTCTGAGTTTGATCACAAGTTTCAGGAGATCGGTTTGGAG     780
L  P  A  D  T  P  Y  S  E  F  D  H  K  F  Q  E  I  G  L  E
AGAGGTTGGGGTGACACAGCCGAGAGGGTGATGGACATGATACAGCTACTACTCGACCTT     840
R  G  W  G  D  T  A  E  R  V  M  D  M  I  Q  L  L  L  D  L
CTCGAGGCTCCTGATTCTTGCACTCTTGAGAAATTCCTCGGCAGGATCCCAATGGTATTC     900
L  E  A  P  D  S  C  T  L  E  K  F  L  G  R  I  P  M  V  F
AATGTCGTAATCCTCTCACCTCATGGTTACTTTGCTCAAGCCAATGTCCTTGGTTACCCT     960
N  V  V  I  L  S  P  H  G  Y  F  A  Q  A  N  V  L  G  Y  P
GATACTGGTGGCCAGGTTGTGTATATCCTCGATCAAGTCCGTGCTTTGGAGAATGAAATG    1020
D  T  G  G  Q  V  V  Y  I  L  D  Q  V  R  A  L  E  N  E  M
CTTCAACGCATCAAGCAACAAGGCCTTGATATTATCCCTCGCATCCTTATTGTATCTCGT    1080
L  Q  R  I  K  Q  Q  G  L  D  I  I  P  R  I  L  I  V  S  R
CTGCTCCCTGATGCAGTGGGAACCACCTGCGGTCAGCGTCTTGAGAAGGTCTTCGGCACT    1140
L  L  P  D  A  V  G  T  T  C  G  Q  R  L  E  K  V  F  G  T
GAACACTCACACATTCTTCGGTCCCTTCAGACCGAGAAGGGAATTGTCAGAAGATGGATT    1200
E  H  S  H  I  L  R  S  L  Q  T  E  K  G  I  V  R  R  W  I
TCCCGATTTGAAGTCTGGCCATACCTTGAGACTTACACGGAGGATGTTGCAAATGAAATT    1260
S  R  F  E  V  W  P  Y  L  E  T  Y  T  E  D  V  A  N  E  I
GCTGGAGAGCTACAGGCCAAACCTGATCTGATCATCGGAAACTACAGTGATGGAAATATT    1320
A  G  E  L  Q  A  K  P  D  L  I  I  G  N  Y  S  D  G  N  I
GTTGCTTCTTTGTTAGCCCACAAACTCGGAGTTACACAGTGTACGATTCGTCACGCTCTT    1380
V  A  S  L  L  A  H  K  L  G  V  T  Q  C  T  I  R  H  A  L
GAGAAGACCAAGTACCCGAACTCAGACATCTATTGGAAGTCATTCGAGGAGAAGTACCAC    1440
E  K  T  K  Y  P  N  S  D  I  Y  W  K  S  F  E  E  K  Y  H
TTCTCTTGCCAATTTACTGCTGATTTAATTGCCATGAACCACACTGACTTCATTATTACC    1500
F  S  C  Q  F  T  A  D  L  I  A  M  N  H  T  D  F  I  I  T
AGTACATTCCAAGAGATTGCTGGAAACAAGGATACTGTTGGACAATACGAGTCTCACATG    1560
S  T  F  Q  E  I  A  G  N  K  D  T  V  G  Q  Y  E  S  H  M
GCTTTTACTCTCCCTGGCCTCTACCGAGTTGTTCATGGAATAGACGTCTTTGACCCCAAA    1620
A  F  T  L  P  G  L  Y  R  V  V  H  G  I  D  V  F  D  P  K
TTCAACATCGTCTCTCCTGGAGCTGATCTGTCTATTTACTTCCCTTACACCGAAGAGAAG    1680
F  N  I  V  S  P  G  A  D  L  S  I  Y  F  P  Y  T  E  E  K
AAAAGACTTAAAGCCCTCCATCCAGAAATTGAAGAGCTTCTCTACAGTGAAGTTCAGAAC    1740
K  R  L  K  A  L  H  P  E  I  E  E  L  L  Y  S  E  V  Q  N
GAAGAACACATATGTGTTCTCAAGGACCGCAACAAGCCAATCATATTCTCGATGGCTAGG    1800
E  E  H  I  C  V  L  K  D  R  N  K  P  I  I  F  S  M  A  R
CTAGACCGAGTAAAGAACATGACTGGCCTTGTCGAGTGGTATGGTAAGAACAAGAAGCTC    1860
L  D  R  V  K  N  M  T  G  L  V  E  W  Y  G  K  N  K  K  L
CGACAGCTCGTAAACCTTGTGGTAGTTGCTGGTGACAGGAGGAAGGAGTCCAAGGACATA    1920
R  Q  L  V  N  L  V  V  V  A  G  D  R  R  K  E  S  K  D  I
GAAGAGAAGGAAGAGATGAAGAAAATGTACGGGCTTATCGAGGAGTACAACTTGAATGGC    1980
E  E  K  E  E  M  K  K  M  Y  G  L  I  E  E  Y  N  L  N  G
CAGTTCAGGTGGATCTCTGCCCAAATGAACAGGGTGAGGAATGGTGAGCTCTACAGGTAC    2040
Q  F  R  W  I  S  A  Q  M  N  R  V  R  N  G  E  L  Y  R  Y
ATTGCAGACACCAAGGGAGCATTTGTTCAGCCTGCTTACTATGAGGCTTTCGGGCTCACA    2100
I  A  D  T  K  G  A  F  V  Q  P  A  Y  Y  E  A  F  G  L  T
GTTGTTGAAGCCATGACCTGTGGGCTGCCCACATTCGCTACCTGCCATGGTGGGCCTGCT    2160
V  V  E  A  M  T  C  G  L  P  T  F  A  T  C  H  G  G  P  A
GAAATCATTGTCAATGGAAAATCTGGCTACCACATTGATCCATACCACGGGGACAAGGCT    2220
E  I  I  V  N  G  K  S  G  Y  H  I  D  P  Y  H  G  D  K  A
GCTGAGCTCCTTGTTGAATTCTTTGAAAAGTCTACGGCTAATCCTTCTCACTGGGAAGCT    2280
A  E  L  L  V  E  F  F  E  K  S  T  A  N  P  S  H  W  E  A
ATTTCTAATGGTGGATTGAAACGAATTGAGGAGAAATACACATGGAAGATCTACTCCGAC    2340
I  S  N  G  G  L  K  R  I  E  E  K  Y  T  W  K  I  Y  S  D
AGGCTGCTCACTCTTGCTGGTGTTTACGGTTTCTGGAAGTACGTCTCAAACCTTGACCGT    2400
R  L  L  T  L  A  G  V  Y  G  F  W  K  Y  V  S  N  L  D  R
CGTGAGGCTCGTCGTTACCTTGAAATGTTCTACGCCCTTAAGTACAAAAAGCCCGCTGAA    2460
R  E  A  R  R  Y  L  E  M  F  Y  A  L  K  Y  K  K  P  A  E
TCGGTACCATTGCTTGTTGAGGATTGAAACTCAAATTCAAACTGAAGATGAGTAGTGTTG    2520
S  V  P  L  L  V  E  D  *
TTGGTGATGAATATGCATGGAAGAGCCATGGAGT                               2554
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Fig. 1: The nucleotide and deduced amino acid sequence of a cDNA of sucrose synthase from *C. rubrum*. The coding region is given in capital letters. Deduced amino acids are shown in one-letter code.

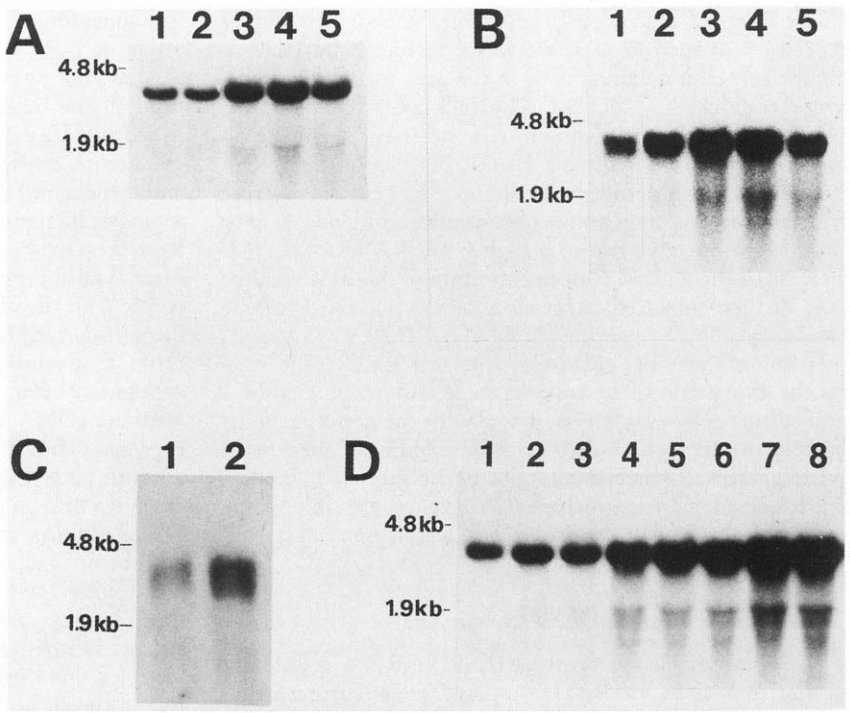

Fig. 2: Northern blot analysis of total RNA from suspension culture cells of *C. rubrum*. Twenty five µg RNA was separated on a formaldehyde-agarose gel that was briefly stained with acridine orange and photographed before blotting onto nitrocellulose filters to assure that equal amounts of RNA were used based on the intensity of the ribosomal RNA bands. The blot was probed with radiolabeled cDNA coding for sucrose synthase (CSS1) from *C. rubrum*. A) Effect of different sugars and mannitol on CSS1 expression. Cells were grown photoautotrophically (1) or incubated for 36 h with 100 mM of mannitol (2), D-glucose (3), D-fructose (4) and sucrose (5). B) Time course of CSS1 expression after addition of 100 mM D-glucose. Samples were removed after 0 h (1), 1 h (2), 12 h (3), 24 h (4) and 48 h (5). C) Effect of 6-deoxy-glucose (6DG) on the expression of CSS1. Cells were grown photoautotrophically (1) incubated with 30 mM 6-DG for 24 h (2). D) Influence of different glucose concentration and mannitol on CSS1 expression. Cells were grown photoautotrophically (1) or incubated for 12 h with 5 mM (2), 10 mM (3), 20 mM (4), 50 mM (5), 100 mM (6), 150 mM (7), and 300 mM (8) of D-glucose.

termination of the activity of acidic and neutral invertases. Neither of the two intracellular invertase activities showed a tissue specific distribution.

Cloning of a sucrose synthase cDNA from C. rubrum and sequence analysis

A cDNA library of *C. rubrum* in lambda gt10 was prepared as described in Material and Methods and was screened under reduced stringency for sequences hybridizing to the potato sucrose synthase cDNA (Salanoubat and Belliard, 1989). One of the positive clones was purified and the 3.0 kb insert subcloned into pUC 19. The complete coding region was sequenced and the 2554 bp long cDNA sequence and the derived amino acid sequence are shown in Fig. 1. It contains one open reading frame starting from nucleotide 75 with an ATG start codon and ending at nucleotide 2484 with an TGA stop codon. The putative translational start site is the only methionine codon between a stop codon (UGA) close to the 5' end of the cDNA and the DNA sequence. In addition the +4 and +5 positions, with respect to the methionine codon, are identical to the proposed consensus sequence of start methionines in plants (Lütcke et al., 1987). The open reading frame encodes a polypeptide chain of 803 residues with a calculated molecular weight of 92.065 kDa.

In addition to the open reading frame the cDNA also contains 75 bp 5' untranslated and about 520 bp 3' untranslated sequence with a poly(A) tail. The consensus signal for polyadenylation AATAAA (Heidecker and Messing, 1986), which is usually located 10 to 30 nucleotides upstream of the poly(A) tail, is located 35 bp upstream of the 3' end of the complete cDNA clone. The calculated pI-value is 6.24.

Expression of sucrose synthase after addition of sugars to photoautotrophic suspension culture cells

To address the question of whether the higher sucrose synthase activity after preincubation with D-glucose, sucrose or D-fructose is due to an increased expression of the corresponding gene, a Northern blot analysis was carried out. Total RNA was isolated from a photoautotrophic culture and a culture that was preincubated in the presence of 100 mM of D-glucose, D-fructose or sucrose for 48 h. The RNA was probed with the radiolabeled 2.2 kb cDNA insert of pSS1 containing the cDNA sequence shown in Fig. 1. Figure 2 A shows that in all samples a single transcript of 3.0 kb could be detected. Preincubation in the presence of 100 mM D-glucose, D-fructose or sucrose significantly enhanced the level of sucrose synthase transcript. To control the effects of osmotica on sucrose synthase expression, suspension culture cells were incubated with equivalent molarities of mannitol. Figure 2 A, lane 2 shows that preincubation in the presence of 100 mM mannitol did not result in an elevated mRNA level compared with the photoautotrophic control cells.

Characterization of the induction of sucrose synthase by sugars

In further experiments the nature of induction of sucrose synthase by D-glucose was determined more precisely. To study the time course of induction by glucose, 100 mM glucose was added to photoautotrophically grown cells and samples removed at different time intervals. Figure 2 B shows that expression of sucrose synthase could already be detected after 1 h; the mRNA level increases up to 12 h and incubation for 48 h results in decreasing RNA levels.

The minimal glucose concentration necessary to induce expression of sucrose synthase was determined by incubating the suspension culture cells in the presence of increasing concentrations of D-glucose. The cells were incubated for 12 h, since this time was shown to be sufficient to induce expression of sucrose synthase. Figure 2 D demonstrates that 20 mM glucose is sufficient to induce the gene for sucrose synthase and that increasing concentrations of glucose up to 100 mM do not give rise to a higher mRNA level. Further increase of the glucose concentration up to 300 mM resulted in a further enhanced expression of the sucrose synthase gene.

To analyze whether glucose or some metabolite of it induces the expression of sucrose synthase, autotrophic suspension culture cells were preincubated with the non-metabolizable glucose analogue, 6-deoxyglucose. Figure 2 C shows that 6-deoxyglucose, which cannot be phosphorylated, induces expression of sucrose synthase to a level comparable to the induction observed in the presence of D-glucose.

Tissue specific expression of sucrose synthase

Expression of sucrose synthase (CSS1) mRNA in different tissues from *C. rubrum* was compared on a Northern blot with equal amounts of total cellular RNA from four different tissues (Fig. 3). Probing of the blot with the radioactively labeled insert of cDNA clone pSS1 reveals that the transcript for sucrose synthase could only be detected in stems, roots and flowers but not in mature leaves. Whereas a low level of sucrose synthase mRNA could be detected in flowers, the level was considerably higher in roots and stems.

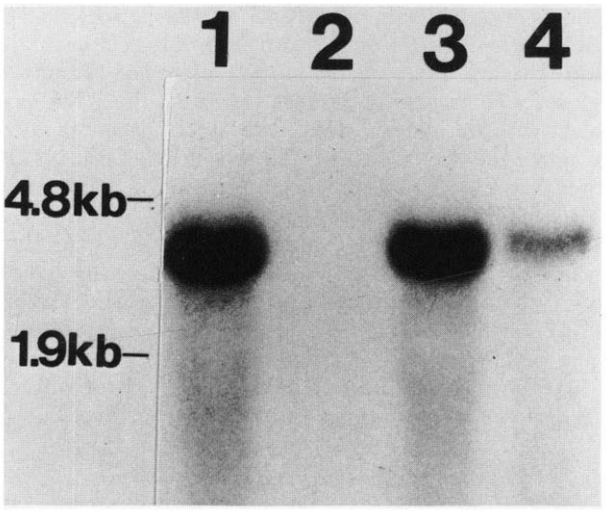

Fig. 3: Northern blot analysis of total RNA from different tissues from *Chenopodium rubrum* plants. Twenty five µg RNA was separated on a formaldehyde-agarose gel that was briefly stained with acridine organe and photographed before blotting onto nitrocellulose filters to assure that equal amounts of RNA were used based on the intensity of the ribosomal RNA bands. The blot was probed with radiolabeled cDNA coding for sucrose synthase (CSS1) from *C. rubrum*. RNA was isolated from stems (1), source leaves (2), roots (3), and flowers (4).

Discussion

A sucrose synthase cDNA from *C. rubrum* (CSS1) has been cloned based on the high sequence homology to published sucrose cDNA sequences. Sequence comparison with all sucrose synthase cDNA sequences found in the EMBL and Genebank DNA data bank showed a high degree of homology; the homology to sucrose synthase cDNA sequences from a variety of monocotyledonous and dicotyledonous plants varied only from 73 % to 77 %. High similarities (84 % to 91 %) are also found between the predicted amino acid sequences derived from the CSS1 cDNA sequence and the corresponding amino acid sequences from other cloned sucrose synthases. From rice, barley and maize two different sucrose synthase cDNA sequences are reported, labeled type 1 and type 2 (Koch et al., 1992). The CSS1 sequence does not show a significantly higher homology to either of the two different types of sequences (73.1 % and 73.6 %, respectively).

The addition of D-glucose, D-fructose or sucrose to photoautotrophically grown cells of *C. rubrum* to initiate mixotrophic growth resulted in a 3 to 4 fold higher sucrose synthase activity, which was paralleled by an elevated level of sucrose synthase mRNA. Control experiments using mannitol ruled out a non-specific induction due to the osmotic effect of the sugars applied. These data show that the sucrose synthase from *C. rubrum* is induced by sugars, as has been reported for sucrose synthase genes from potato (Salanoubat and Belliard, 1989), rice (Karrer and Rodriguez, 1992) and bean (Heim et al., 1993). These data provide additional support for the function of sugars in regulating gene expression in plants. There is increasing evidence that sucrose or specific hexoses are important for metabolic regulation and growth control in lower (Hilgarth et al., 1991) and higher plants (Williams et al., 1992).

Photosynthetic genes and chlorophyll synthesis are repressed (Sheen, 1990; Krapp et al., 1991; Schäfer et al., 1992) whereas a number of genes are induced by sugars. Most of the proteins encoded by sugar induced genes are involved in storage processes or other sink tissue specific enzyme reactions (Müller-Röber et al., 1990; Visser et al., 1991; Hilgarth et al., 1991; Shih and Goodman, 1988). This observation shows that sugars are not only used as carbon source for heterotrophic growth but they also function as molecular signals to coordinately induce a number of sink specific enzymes. In addition to the family of sugar inducible genes, sucrose synthase genes from *Arabidopsis* (Martin et al., 1993) and maize (Koch et al., 1992) were found to be repressed by sugars. The occurrence of differentially regulated sucrose synthase genes may be explained by the diverse metabolic processes involving sucrose synthase activities and the data presented in this paper may indicate that the sugar induced genes are the ones important for sink metabolism. This is further supported by transgenic potato plants expressing a sucrose synthase antisense construct. The decreased activity of the sugar inducible potato sucrose synthase resulted in a dramatically decreased yield (Sonnewald et al., 1994).

In further experiments the induction of sucrose synthase in *C. rubrum* suspension culture cells has been characterized in more detail. The amount of sucrose synthase mRNA in autotrophic cells from *C. rubrum* is induced to a consider-

ably higher level above a concentration of 20 mM glucose. Further increase of the sugar concentration up to 100 mM did not significantly change the mRNA level. Although the sucrose synthase expression is further elevated above 100 mM, it remains to be determined whether this constitutes an osmotic stress reaction rather than being related to specific induction of sink metabolism. In previous reports glucose concentrations of 100 mM to 400 mM were necessary to induce sucrose synthase expression in cut plant tissues incubated in sugar solutions (Salanoubat and Belliard, 1989; Koch et al., 1992; Karrer and Rodriguez, 1992). The significantly lower sugar concentrations sufficient in the suspension culture system may be related to the fact that in the suspension culture system the individual cells are exposed to the exogenous sugars that are immediately taken up by constitutively expressed (Roitsch and Tanner, 1994) high affinity glucose transporters (Gogarten and Bentrup, 1986).

The finding that sucrose synthase expression is elevated as early as 1 h after addition of glucose to photoautotrophic cells of *C. rubrum* may indicate that this enzyme activity is an important early step for the source/sink transition in plants and thus for the establishment of metabolic sinks.

It has been suggested that one major function of sucrose synthase in sink tissues is the production of precursors for starch biosynthesis (Chourey and Nelson, 1976). This is supported by the correlation between the time course of sucrose synthase expression and the starch content of *C. rubrum* suspension culture cells shifted to mixotrophic growth by adding glucose. The results presented in this paper show that the sucrose synthase expression increases up to 12 h after the addition of glucose and declines to lower rates after 24 h which correlates with the starch content reported for similar experiments with *C. rubrum* suspension culture cells by Schäfer et al. (1992). The inducing sugars could be substituted by the non-metabolizable glucose analogue, 6-deoxyglucose. This observation indicates that the hexose sugars and not a metabolite thereof is the primary signal to induce sucrose synthase expression. These data and the characterization of a highly active extracellular invertase in *C. rubrum* (Roitsch et al., 1995) suggest that the observed induction of sucrose synthase by sucrose is due to hydrolysis of sucrose and transcriptional activation by glucose. The proposed mechanism suggests that hexoses may also be the actual signal in systems where sucrose was found to induce enzymes of heterotrophic metabolism (Salanoubat and Belliard, 1989; Müller-Röber et al., 1990). The finding that the sugar inducible promoter of the potato proteinase inhibitor II, which is not related to carbohydrate metabolism, is not induced by 6-deoxyglucose (Kim et al., 1991) may indicate that different sugar induction pathways operate in parallel in plant cells. The data presented suggest that in *C. rubrum* the non-phosphorylated glucose interacts with a putative intracellular or extracellular receptor, which ultimately results in activation of genes that are involved in sink metabolism. Although this is the first report demonstrating that the initial signal for metabolic regulation in plants is the non-phosphorylated glucose, the finding that an extracellular invertase from *C. rubrum* is also induced by 6-deoxyglucose supports the proposed signal transduction pathway (Roitsch et al., 1995).

In *C. rubrum* plants the tissue specific distribution of sucrose synthase activity correlates with the mRNA levels. The specific expression of sucrose synthase in sink tissues of *C. rubrum* plants supports the use of sucrose synthase activity or the level of sucrose synthase mRNA as a biochemical marker for sink strength, as has been suggested before (Claussen et al., 1985; Sung et al., 1989; Wang et al., 1993). The finding that sucrose synthase activity in different tissues of *C. rubrum* plants is 200 to 1270 fold higher than the soluble invertase activities suggests that intracellular sucrose hydrolysis via sucrose synthase is the dominating pathway. The highest level of both sucrose synthase activity and mRNA has been found in stems, which supports a specific function in the vascular system, as has been suggested previously (Lowell et al., 1989). It remains to be determined whether the observed sink tissue specific expression is only due to an upregulation by sugars or whether the promoter is, in addition, tissue specifically regulated. The much lower expression of sucrose synthase in flowers would favour the latter hypothesis, but a detailed promoter analysis in transgenic plants will be required to answer this question.

The correlation between sink tissue specific expression in plants and sugar induced expression in the suspension culture system demonstrates that the photoautotrophic culture used in this study is an appropriate model system to analyze the source sink transition in higher plants. Further studies will be necessary to elucidate the signal transduction pathway mediating sugar regulated gene expression and to determine the specific function of sucrose synthase in sink organs.

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